THE ESSENTIAL OIL

of

ACRADENIA FRANKLINII

by

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My colleague, Mr. J. D. Stevens, was also working on Essential oils, and I now record my appreciation of his help and suggestions during the year.

Finally, I wish to express my thanks to other members of the Staff of the Chemistry Department for their interest and encouragement throughout the year.

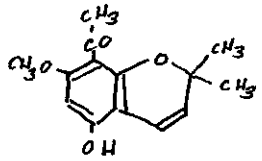
Mary E. Pitman

Mary E. Pitman.

CONTENTS.

INTRODUCTION

The C. S. I. R. O. isolated two crystalline compounds from the leaves of Acradenia Franklinii, a shrub endemic to Tasmania. These two compounds, designated A and B, were investigated by Dr. A. A. Komzak of the University of Tasmania, and he showed that Compound B was identical with Methyl allo evodionol, which occurs naturally in the Queensland tree Evodia elleryana which grows in the region round Mackay.



Methyl allo evodionol.

Komzak also isolated a small amount of a third crystalline compound, C, and made some preliminary investigation on it.

The work undertaken this year was an attempt to find further information about the nature of Compound A, and also Compound C. The chief difficulty in this work was the isolation of the two compounds, more particularly A, in sufficient quantities.

This thesis is divided into five sections.
Section I deals with the general properties and extraction of the essential oil of Acradenia Franklinii.
Section II deals with the isolation of Compounds A and C from the essential oil of Acradenia Franklinii.
Section III contains a summary of the known information on Compound A and an account of the oxidation of Compound A.
Section IV contains an account of the structure of Compound C.
Section V deals with the erection of a high efficiency fractionating column.

SECTION I.

The Essential Oil of Acradenia Franklinii.

Acradenia Franklinii is a shrub of 8 to 12 feet in height endemic to the West Coast of Tasmania, where it grows on the banks of the Gordon, Pieman and Franklin Rivers. The material for this investigation all came from the region round the Gordon River. The leaves and the essential oil obtained from them both have a characteristic bitter smell.

Leaves and twigs weighing 509 lb. yielded 1658 g. oil on steam distillation.

The oil has the following constants:

d_4^{20}	0.899
n_D^{20}	1.4926
α_D^{20}	-24.486^0
Acid No.	5.145
Ester No.	16.45
Ester No. after Acetyln.	35.08
% Carboryl Content	9.25

(Calc. as Cpd. A_3 M.W. 314)

Experimental

Steam Distillation of Plant Material

The system used for the steam distillation of the plant material is shown in Fig. 1. The drum used for the distillation had the following dimensions: r = 19", h = 63". It was fitted with a wooden lid lined with galvanised iron, and a seal was formed between the lid and the drum by sticking a length of soft rubber tubing round the top edge of the drum. This seal was not entirely satisfactory, and had to be renewed before each distillation. It was necessary to increase the weight of the lid during distillation, and this was done by stacking

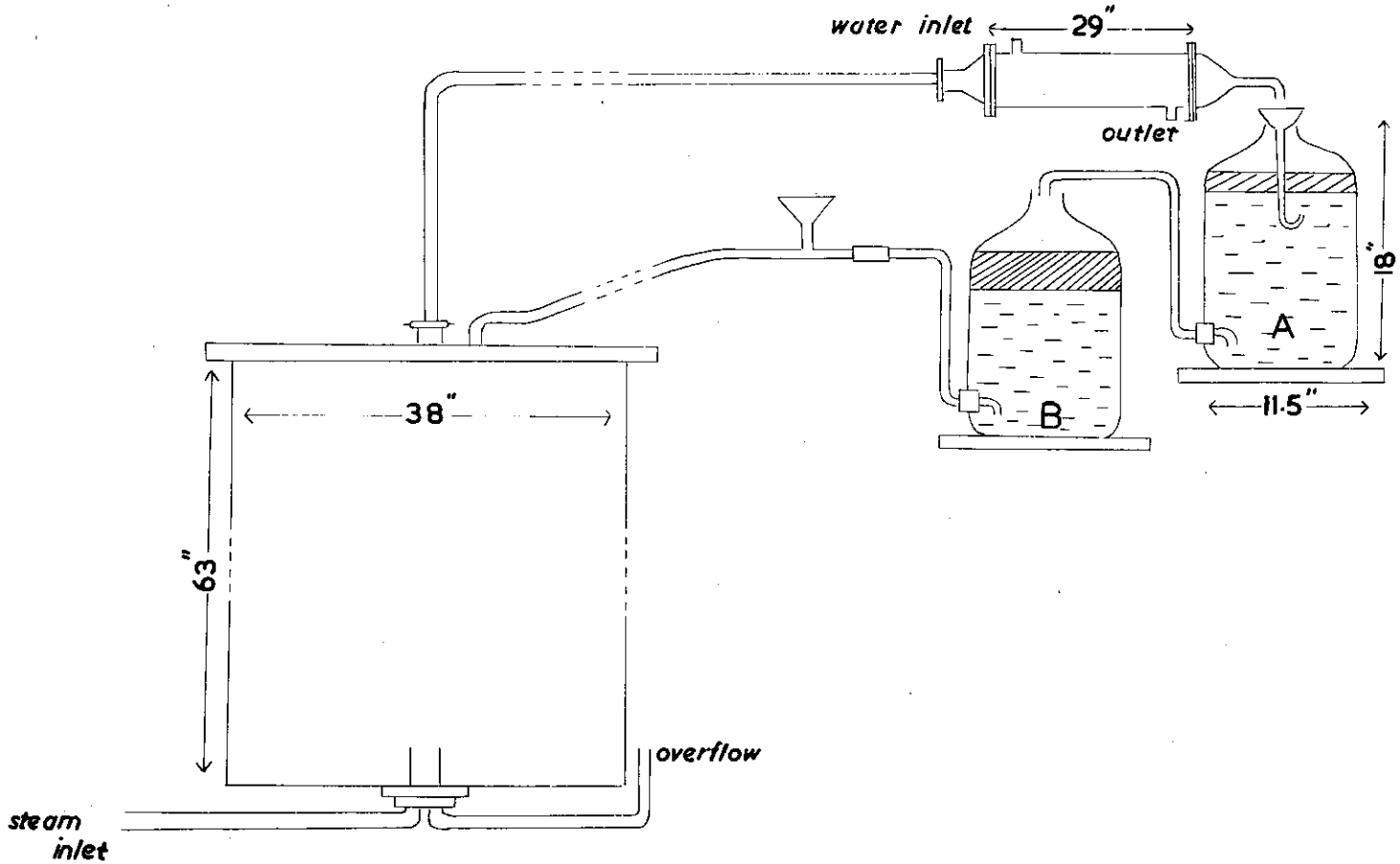


FIGURE 1

bricks evenly over the surface of the lid. Excess water in the drum was removed by an overflow pipe inserted at the bottom. The vapours from the distillation drum were condensed in a large Liebig type copper condenser, 29" in length.

Most of the oil in the distillate was collected above the aqueous phase in the marriot bottle A. Marriot bottle B contained 3 litres of petroleum ether (B.P. 40-60) to extract any oil coming over in the aqueous phase from bottle A. the aqueous phase from bottle B was returned to the distillation drum to ensure complete recovery of the oil. A plastic funnel was inserted in the line returning the aqueous phase to the drum to act as a "surge tower".

A total of 509 lb. of leaves and small twigs of Acradenia Franklinii were distilled in two batches of approx. 250 lb. The leaf material was placed in the drum and steam passed through it from a boiler with gauge reading from 26 to 30 lb/sq.in. Distillation was continued until no oil could be seen floating on the surface of a sample withdrawn from the distillate. The distillation took 13 days for each batch.

When the distillation was complete, the aqueous layers in bottles A and B were siphoned off until the oil layer and petroleum ether layer respectively reached the top of the siphon outlet. The oil from bottle A was then diluted with ether and the remainder of the aqueous phase removed in a 2 litre separatory funnel. The ether phase was dried with anhydrous sodium sulphate, and the ether removed by distillation at ordinary pressure followed by heating to 60° under water vacuum pressure. This operation was to ensure the complete removal of the ether. The petroleum ether solution from bottle B was separated and dried, and the petroleum ether removed by distillation up to 80°.

The total yield of oil was 1658 g., which represents a yield of .716% oil from the leaf material.

Determination of Physical Constants

The oil used for the determination of physical and chemical constants was dried over anhydrous sodium sulphate and clarified by filtration through a pad of filter earth (Mollicel 250).

1. Density

The density was determined with a Fisher-Davidson type densitometer (1, p.32). This instrument gives the density of a liquid directly at 20°. The value obtained was $d_4^{20} = 0.899$.

2. Refractive Index.

The R. I. of the oil was determined with an Abbe refractometer (2,p.244). Normal daylight was used for illumination. The value obtained was $n_D^{20} = 1.4926$

3. Optical Rotation (2, p.240)

The optical rotation was determined with a Lippich double-field type polarimeter. As the oil is a dark reddish brown in colour, it was necessary to use a 50 mm. polarimeter tube for the determinations, which were made at 20°. The rotation is reported as optical rotation determined in a 100 mm. tube, according to convention. This value is twice the rotation observed using the 50 mm. tube. $\alpha_D^{20°} = -24.48$

Determination of Chemical Constants.

1. Acid Number. (2, p.263)

The acid number is defined as the number of milligrams of potassium hydroxide required to neutralize the free acids in 1 g. oil. The acid number was calculated by the equation:

$$\text{Acid No.} = \frac{5.61 \times 1.05 \times a}{S} \quad \text{where}$$

a = no. of ml. of 0.105 N sodium hydroxial solution used for the neutralization.

S = wt. of oil sample in grams.

Wt. of Sample	Titre(a)	Acid No.
2.108	1.85	5.17

Average Acid No = 5.145.

About 2 g. oil were weighed accurately in a 10 ml. beaker, and then transferred to a 250 cc. r.b. quick-fit flask using 15 ml. of neutral 95% ethanol. 8 drops of 1% phenolphthalein solution were added, and the free acids titrated with 1.105 N aqueous sodium hydroxide solution . During neutralization a further 15 ml. of neutral ethanol were added to prevent emulsification of the oil. The first appearance of a red colouration which did not fade within 10 secs. was considered the end point. This was difficult to determine accurately owing to the natural colouration of the oil.

2. Ester Number (2, p.265)

The ester number is defined as the number of milligrams of potassium hydroxide required to saponify the esters present in 1 gm. of oil.

The ester number was calculated using the equation

$$\text{Ester No.} = \frac{28.05 \times a}{S}$$

$$= \frac{28.05 \times b \times 0.445}{S \times 0.5} \quad \text{where}$$

a = no. of ml. of 0.5N NaOH solution used in the saponification
b = the difference in the titre of 0.455 N HCl for the blank and for the saponification.
S.= wt. of oil sample in grams.

Wt. of Sample	Titre Diff(b)	Ester No.
2.067	1.35	16.3
2.108	1.40	16.6

The average Ester No. = 16.45

To the neutralized oil from the Acid No. determination were added 10 ml. of 0.5N alcoholic NaOH solution. A water cooled reflux condenser was attached to the flask and the contents were refluxed for 1 hr. on a steam bath. The

solution was cooled for 15 minutes at room temperature and the excess alkali titrated with 0.445 N HCl, after adding 3 more drops of phenolphthalein solution. Two blank determinations were carried out under the same conditions, but omitting the oil.

3. Ester No. after Acetylation. (2, p.277)

The ester number after acetylation was determined as a measure of the alcohol content of the oil. It was determined by the acetyl chloride-dimethyl aniline method which is useful for the determination of tertiary sesquiterpene alcohols. As it is not known which terpene alcohols are present in Acradenia oil, the results of this determination are reported as

(i) Ester no. after acetylation, using the same formula as the ester no., but here S no. of grams of acetylated oil used for the determination.

(ii) Percentage of free alcohol (calculated as sesquiterpene alcohol, (M.W. = 222) in the original oil using the equation

$$\% \text{ alcohol (M.W. 222)} = \frac{222 \times d}{561.04 - 0.42\,d.}$$ where

d = ester no. after acetylation - ester no.

Wt. of Sample (S)	Titre(b)	Ester No. after Acetyln.
2.102	2.95	35.04
2.133	3.00	35.12

Average ester no. after acetylation = 35.08 = 35.1.

% alcohol content in oil of M.W. 222 = 7.42.

10 ml. of oil was placed in a 250 ml. r.b. flask fitted with a glass stopper, and the flask was cooled in an ice-water mixture. 20 ml. of purified dimethyl aniline were added and the contents of the flask mixed thoroughly. Then 8 ml. of acetyl chloride and 5 mil. of acetic anhydride were added, and the mixture left to stand for $\frac{1}{2}$ hr. at 20°, and then for 4 hrs. at 36° in a water bath. The acetylated oil, which was blue in colour was then washed 3 times with 75 ml. portions of ice water,

followed by successive washes with 25 ml. portions of 5% H_2SO_4 until the separated acid layer failed to liberate dimethyl aniline on addition of excess caustic soda solution. Six washes were made, and these also removed the blue colour from the oil. The acetylated oil was then washed with 10 ml of 10% Na_2CO_3 solution and finally washed neutral with water. The oil was dried over anhydrous sodium sulphate and the ester no. determined as before.

A "blank" carried out by first adding acetic anhydride and acetyl chloride to dimethyl aniline and then standing for 4 hrs. at 36° also turned blue. Thus the colouration was not due to any constituent of the oil.

4. Carbonyl Determination.

This was determined by the "Standard Procedure" detailed in (2, p.235). This method is based on the reaction of hydroxylamine hydrochloride with carbonyl Compounds, and determines the quantity of HCl liberated by the reaction. Compounds A, B and C are all carbonyl compounds, but as Compound A appears to occur in greater amounts than either B or C, the percentage of carbonyl compound was determined in terms of A. The actual extent of the occurrence of A is probably a good deal less than the figure obtained by this means.

$$\% \text{ Carbonyl Compounds} = \frac{m \times a \times 0.494}{20 \times S.}$$

a = no. of ml. of alc. NaOH used for the neutralization
S = wt. of oil sample in grams.
m = M.W. of A = 314.

Wt of Sample (S)	Titre(a)	% Carbonyl
1.358	0.80	9.14
1.571	0.95	9.37

Average % Carbonyl Value = 9.25.

About 1.5 g. of oil were weighed accurately ain a 100 ml. conical flask and 35 ml. of 0.5N hydroxylamine hydrochloride solution were added. The flask was left to stand at room temperature for 24 hrs. and the liberated HCl was then titrated with 0.494 N alc. NaOH solution. The titration was continued until the original greenish shade of the hydroxylamine hydrochloride solution was obtained. A flask containing 35 ml. of hydroxylamine hydrochloride solution was used as a blank to assure an accurate colour match.

All the Solutions used for the determination of Chemical constants were prepared by Mr. Stevens.

SECTION II

Isolation of Compounds A and C from the essential oil of Acradenia Franklinii

Compound A occurs in the oil of Acradenia Franklinii to the extent of about 3%, this figure being based on the maximum yields of the compound so far obtained.

The method of isolation of compound A used by the C. S.I.R.O. was not practical on a large scale, as it involved extraction of the leaf material with methanol and the working up of the methanolic extract and a large extractor was not available. As compound A was known to be steam volatile, from the work of Dr. Komzak, a large supply of acradenia essential oil was obtained by steam distillation of Acradenia leaves.

Chromatography of the essential oil as a method of isolation of compound A only yielded satisfactory results when Lights alumina was used. Dr. Komzak found that using this, satisfactory yields could be obtained, while if B.D.H. alumina was used the result was unsatisfactory and the fractions generally

failed to crystallize. It was also found that May and BBakers' alumina did not yield satisfactory results. Thus it seems that the activity of the aluminium oxide used for the chromatography is a critical factor in the separation of compound A by this means.

As no Lights alumina was available, an attempt was made to isolate compound A with the use of Girard reagents. A is a carbonyl compound, and Girard reagents form water solubles derivatives with carbonyl compounds which are insoluble in non-hydroxylic organic solvents, and so relatively small quantities of carbonyl compounds can be removed from natural products such as essential oils.

Reagent T is preferable for isolation purposes owing to its greater solubility than reagent P (3) and the first intention was to use reagent T for the isolation, but this had to be abandonned. Reagent T is usually prepared from tremethylamine according to the reactions in equations 1) and 2)

(1) $N\,Me_3 + ClCH_2.COOC_2H_5 = Me_3N.CH_2.COOC_2H_5\ {}^{+}Cl^{-}$.

(2) $Me_3N.CH_2.COOC_2H_5\ {}^{+}Cl^{-} + H_2N.NH_2 = Me_3N.CH_2.CO.NH.NH_2\ {}^{+}Cl^{-}$

Reagent T.

As trimethylamine was not easily obtainable in large quantites as a starting material for the preparation, an alternative method of preparation was tried.

The possibility of obtaining the inter mediate I by esterfication of betaine hydrochloride, $Me_3N^{+}CH_2\ COO.H\ Cl^{-}$ was investigated, but an attempt to esterify betaine hydrochloride by the Fisher Speier method failed completely. Another alternative would be to prepare Reagent T via the acid chloride. This would require the acid chloride of betaine hydrochloride as a starting material. Thionyl chloride would be a suitable reagent

for converting betaine to its acid chloride, as the products of the reaction can easily be removed. Betaine hydrochloride was found to be insoluble in thionyl chloride, and also insoluble in pyridine and in dioxane, the two other most suitable solvents for the reaction. Therefore the idea was rejected for lack of a suitable solvent. This must be non-polar as a polar solvent would dissociate the acid chloride as soon as it was formed. Therefore Reagent P was used for the extraction. This had the advantages that it was relatively simple to prepare and more stable than Reagent T and therefore could be kept. Some preliminary experiments were made on acetophenone as a type compound to determine the most favourable conditions of reaction. At first the procedure of Girard and Sandulesco (4) was used (Expts. 1-3) but this did not yield satisfactory results. The products of the extraction were liquid, and so oximes were prepared for identification purposes. Their method was not very precise as to the amount of reagent to be used, and variations in the excess of reagent and the volume of solution did not have any effect on the yield. Therefore this method was discarded in favour of a procedure suggested by Dr. Polya and the dinitrophenylhydrazone prepared for identification purposes. This gave good results with acetophenone, 82% of the acetophenone being extracted by the Reagent P. The only significant differences in the method used were a decrease in the volume of solution for the reaction, the rapid cooling of the solution after refluxing, and the addition of ice to the Na_2CO_3 solution. Thus it appears that rapid cooling and neutralization of the acetic acid in the cold are important factors.

The method was applied on a small scale to compound B (Expt. 6) finally to 100 mg. of Compound A (Expt. 7) 44 mg. of A were extracted by the reagent P. Even though this was only a 44% yield from the pure compound, the method was applied to Acradenia oil at this stage in the hope that adequate yields would be obtained when carried out on a large scale. Two Girard extractions were made on two 100 g. lots of Acradenia oil (Expts. 8 and 9). The products of the two extractions remained liquid, but on chromatographing on alumina 0.389 g. and 0.693 g. of crude compound A were obtained (Columns 2 and 3). These were unsatisfactory yields for isolation purposes and not quantitative. The lou yields may be due to the structure of compound A. According to Girard and Sandulesco (4) the rate of hydrazone formations is a function of the structure of the ketone, and they arranged the various types in the following approximate order of decreasing reactivity:

methyl alkyl > alicyclic > methyl aryl > diaryl.

It has also been observed that an accumulation of substitutents adjacent to the carbonyl group also reduces the reactivity considerably. The proposed structure for compound A contains a methyl aryl ketone with two adjacent substituents. The reaction of carbonyl groups with Girard reagents is rapid, but additional time was allowed in refluxing when extracting compound A to ensure the completeness of the reaction. In the preliminary experiments with acetophenone it was found that additional reflux time did not significantly affect the yield.

It was decided to remove some of the lower boiling fractions of the oil by distillation and to work on a more highly concentrated oil. 612 g. of oil were concentrated to 99.5 g. by distillation and 35 g. of the residue were chromatographed on an alumina column. The only crystalline product obtained was 35 mg. of compound C.

The remaining 64 g. of the residue were treated with Reagent P and 6.14 g. of a liquid extract was obtained. This extract was never chromatographed, as a more efficient means of separation became available.

At this stage in the work $2\frac{1}{2}$ kilos of Lights alumina was obtained and a preliminary chromatogram of 10 g. of oil yielded 288 mg. of crude compound A. As this was a far more efficient method of extraction than the Girard Rt. P, two 30 g. lots of Acradenia oil were chromatographed on Lights alumina, and yielded a total of 2.93 g. of crude material, m.pt. 112-116° (Columns 5, 6, and 7). As several recrystallizations failed to raise the melting point, it was thought that an additional substance must be present. This proved to be compound C as chromatography of the crude product yielded 101mg. of compound C in addition to 1.959 g. of compound A.

From all isolation methods a total of 3.04 g. of compound A was obtained, which on purification by recrystallization yielded 2.87 g. of A, m.pt. 127°, suitable for oxidation purposes, and this, together with compound A left by Komzak was sufficient to continue the structural investigation.

Experimental

A small chromatogram of 10 g. of Acradenia oil was run on 300 g. of May and Baker alumina to determine qualitativity whether this alumina was suitable for isolation of compound A by chromatography. The oil was dissolved in Petroleum ether (B.P. 40 - 60°) andthe column, which had diameter 17 mm., was made up in Petroleum ether. Benzene, chloroform, and finally methanol were used as eluents. As none of the fractions of eluate crystallized after the solvent was removed, it was concluded that May and Baker's alumina was unsuitable.

Attempted Esterification of Betaine HCl. (5)

Betaine hydrochloride (35 g.) and absolute ethanol (50 ml.) were placed in a 100 ml. bolt-neck flask which was fitted with a two-holed rubber stopper carrying an inlet tube through which HCl gas could be passed through the flask, and a reflux condenser fitted at the top with a calcium chloride tube. The HCl gas was prepared from NH_4Cl and conc. H_2SO_4 in a Kipps apparatus and dried by passing through conc. H_2SO_4. The flask was heated on a sand bath so that the solution boiled gently and a current of HCl gas was passed through the reaction mixture. The solution was refluxed for 3 hours and left overnight. The solid was filtered off and refluxed with acetone to remove any ester. On evaporation the acetone solution gave no residue. The solid residue was dried and found to have m.pt. 226°. Yield 20 g. A small amount of solid was also recovered from the alcohol solution; this also had m.pt. 226°.

M.pt. Betaine hydrochloride: 227-228° (Theor.)

M.pt. $Me_3N^+CH_2.COOC_2H_5$ Cl^- : 143-145° (Theor.)

Thus the product of the reaction was the starting material, betaine hydrochloride.

Preparation of Girard Reagent P (4, 6)

Ice-cold pyridine (316 g. = 322 ml.), which had been dried over KOH and freshly distilled (B.Pt. 114-116°) was added to an ice-cold solution of ethyl chloroacetate (492 g. = 424 ml.) in 1 l. of absolute alcohol. The mixture was allowed to warm up to room temperature, and then heated on a water bath till a test portion no longer smelt of ethyl chloroacetate on acidification. This required $2\frac{3}{4}$ hours. A wt. for wt. solution of hydrazine hydrate (400 g.) was added in small amounts with shaking, and the reaction mixture slowly solidified. The crystalline carbohydrazido methylpyridinium chloride was filtered in a Büchner funnel, washed with alcohol and dried.

Yield: 522 g. (70%) of m.pt. 194°.

The reagent was recrystallized once from ethanol and 400 g. of m.p. 199° was obtained.

M.pt. (theor.) 200°.

$$Cl.CH_2.COOEt. + C_5H_5N = [C_5H_5N.CH_2.COOEt]^+ Cl^-$$

$$[C_5H_5N.CH_2.COOEt]^+Cl^- + H_2N.NH_2 = [C_5H_5N.CH_2.CO.NH.NH_2]^+Cl^- + EtOH.$$

Girard and Sandulesco's Procedure for Extraction with Girard Rts. (4)

The substance under examination was dissolved in EtOH containing exactly 10% acetic acid. 5 to 10% of the reagent was added and the mixture refluxed for 1 hr. After cooling, the solution was poured into ice water containing sufficient Na_2CO_3 to neutralize 0.9 of the AcOH, the pH being checked with bromothymol blue. The soln. was extracted with ether (Extract I) to remove non-carbonyl compounds. To regenerate the ketones the soln. was made 0.5 N in HCl and after standing 1 hr., was again extracted with ether to remove the regenerated ketones (Extract II).

Both ether extracts were dried with anhydrous sodium sulphate and the ether removed by evaporation, leaving Residue I and II respectively.

Experiment I.

Reagents: Acetophenone (1 g.) EtOH (450 ml.) containing 10% AcOH
Girard Reagent P (10 g.)

The Reaction was carried out according to the procedure of G and S. Residues I and II were both liquid, and so oximes were prepared for identification purposes (1, p. 202)

Residue I: Yield of oxime : 0.923 g. m.pt. 57°

Residue II: Yield of oxime : 0.120 g. m.pt. 57°.

M.Pt. Acetophenone oxime (theor.): 60°

Thus only 10% of the acetophenone was extracted by the Rt. P.

Experiment 2.

Reagents: Acetophenone (1 g.) EtOH containing 10% AcOH (150 ml.) Girard Reagent P. (5 g.)

The procedure of G and S. was used but the time of reflux increased to 2 hours.

Residue I : Yield of oxime: 0.945 g. m.pt. 58°

Residue II: Yield of oxime: 0.103 g. m.pt. 56°

Thus again only approx. 10% of the acetophenone was extracted by Rt. P.

Experiment 3.

The reagents used were all carefully dried before use.

The AcOH was dried by refluxing with acetic anhydride for $\frac{1}{2}$ hour.

Reagents: Acetophenone (1 g.) EtOH (spectographic) containing 10% AcOH (200 ml.) Girard Reagent P (10 g.)

The procedure of G and S was used, but the time of reflux was 1 hr. and the procedure for neutralization was different.

A saturated soln. of Na_2CO_3 was made up and chilled in the refrigerator. The reaction mixture was also cooled in the refrigerator and the Na_2CO_3 soln. was added rapidly till the indicator changed colour.

Residue I: Yield of oxime 0.928 g. m.pt. 57°

Residue II: Yield 0.163 g., failed to form an oxime.

Experiment 4.

A new method of procedure was adopted.

Soln. a = AcOH (6 g.) in 99% EtOH (70 ml.)

Girard Rt. P (3.0 g.) was dissolved to 60 ml. with Soln. a. To this was added acetophenone (1.25 g.), and the mixture refluxed for 1 hr. The reaction mixture was cooled in a freezing mixture and poured into a mixture of anhydrous Na_2CO_3 (4.0 g.) dissolved to 100 ml. in H_2O, and crushed ice (500 g.)

The mixture was extracted thoroughly with ether (Extract I). To regenerate the ketone, 65 ml. conc. HCl were added, and the soln. left to stand for 1 hr. The regenerated ketone was extracted with ether (Extract II)

The ether extracts I and II were dried with anhydrous Na_2SO_4 and evaporated to dryness to give Residues I and II respectively. The ketones in Residues I and II were identified as dinitro phenylhydrazones, prepared as follows:-

2, 4 dinitrophenylhydrazine (5 g.) was dissolved in 85% H_3PO_4 (60 ml.) on a water bath and diluted to 100 ml. with 95% EtOH and the soln. filtered. The residue from ether extraction was dissolved in EtOH (20 ml.) and to this was added 20 ml. of D.N.P. soln. The soln. was left to stand and the dinitro phenylhydrazone formed removed by filtration.

M.Pt. Acetophenone dinitrophenylhydrazone (theor.): 250°

Residue I: Yield of dinitrophenylhydrazone 0.2704 g. m.pt. 248°

Residue II: Yield of dinitrophenylhydrazone 2.583 g. m.pt. 247°

Thus the Girard Rt. P. extracted 82% of the acetophenone.

Experiment 5.

The extraction was carried out on a small scale.

Reagents: Acetophenone (125 mg.) Girard Rt. P (300 mg.) dissolved to 6 ml. with Soln. a., Na_2CO_3 (400 mg.); ice (50 g.), conc. HCl (6.5 ml.)

Procedure as in Expt. 4.

Residue I: Yield of dinitrophenylhydrazone 30 mg., m.pt. 249°

Residue II: Yield of dinitrophenylhydrazone 254 mg., m.pt. 247°

This represents an 80% extraction by Rt P.

Experiment 6.

Reagents: Methylalloevodionol (75 mg.), Girard Rt. P (87 mg.) dissolved to 6 ml. with Soln a., Na_2CO_3 (400 mg.), ice (50 g.) conc. HCl (6.5 ml.)

Procedure as in Expt. 4, but time of reflux was 2 hours to ensure complete reaction.

Residue I: Yield 38 mg. m.pt. 105°

Residue II: Yield 33.8 mg. m.pt. 106°.

M.Pt. Methylalloevodionol: 107-8°.

45% of methylalloevodionol (Cpd. B) was extracted by the Girard Rt.

Experiment 7.

The extraction was carried out on a small scale on Cpd. A to test whether Cpd. A would react with the Rt. P.

Reagents: Cpd. A (100 mg.), Girard Rt. P (100 mg.) dissolved to 6 ml. with Soln. a, Na_2CO_3 (400 mg.), ice (50 g.), conc. HCl (6.5 ml.)

Procedure as in Expt. 4., but time of reflux was 2 hrs.

Residue I: Yield 45 mg. m.pt. 125°

Residue II: Yield 44 mg. m.pt. 126°

M.Pt. of Cpd. A: 127°

44% of Cpd. A was extracted by the Girard Rt. P.

Experiment 8.

From Pl Smith's data on the chromatography of acradenia essential oil, from 20 g. oil a total of 1.57 g. of Cpd A and B were obtained, and so it was assumed there would be approx. 10 g. ketonic cpds. in 100 g. oil, and thus approx. 15 g. Girard Rt. P were necessary.

Reagents: Acradenia oil (100 g.) Girard Rt. P. (15 g.) dissolved to 420 ml. with Soln. a, Na_2CO_3 (28 g.) H_2) (700 ml.) ice (3500 g.) conc. HCl (420 ml.)

Procedure as in Expt. 4. Time of reflux 2 hrs.

From Extract I 81.5 g. of oil were recovered.

Residue II: Yield 6.597 g. of an oil which failed to crystallize after seeding and cooling, and so the ketonic components were separated by chromatography (Column 2)

Column 2.

6.597 g. of oil from Expt. 9 were dissolved in Petroleum Ether BP 40-60) and put through a chromatograph column of diameter 17 mm. containing 300 g. of B.D.H. alumina in Petroleum Ether (B.P. 40-60). The eluate was removed with a fraction cutter in approx 20 ml. fractions. The solvent was removed by evaporation and the residue weighed at intervals and transferred to small flasks to crystallize. Details of the column are given in Table I and Fig. 2A.

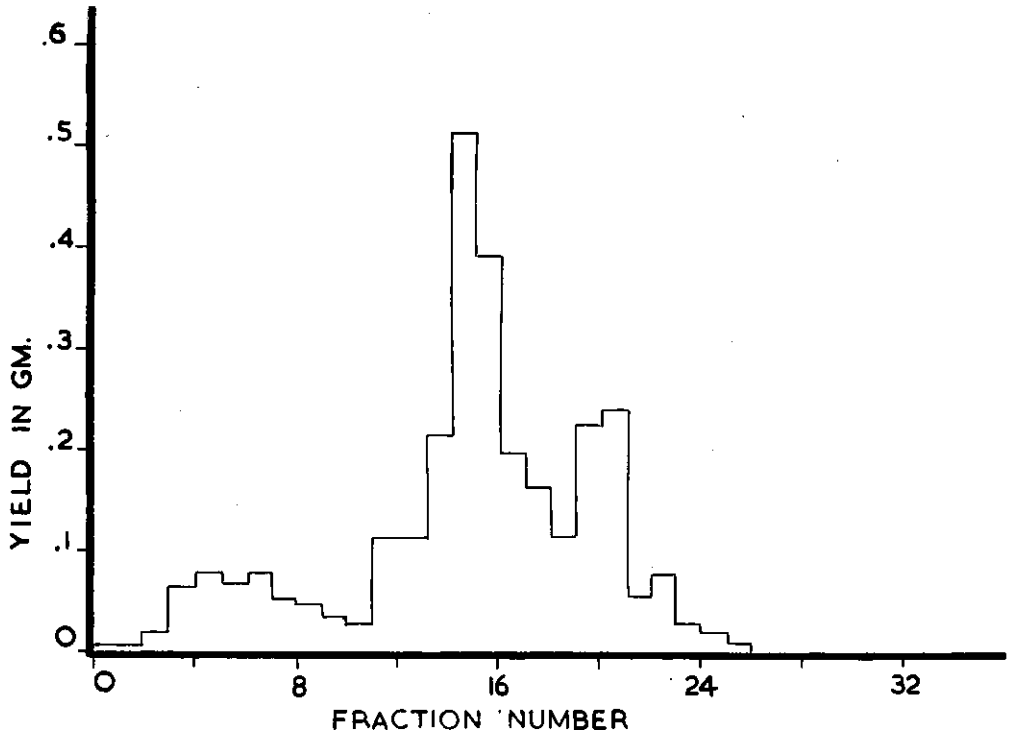

.6
.5
.4
.3
.2
.1
0
YIELD IN GM.
0
8
16
24
32
FRACTION NUMBER

FIG. 2A

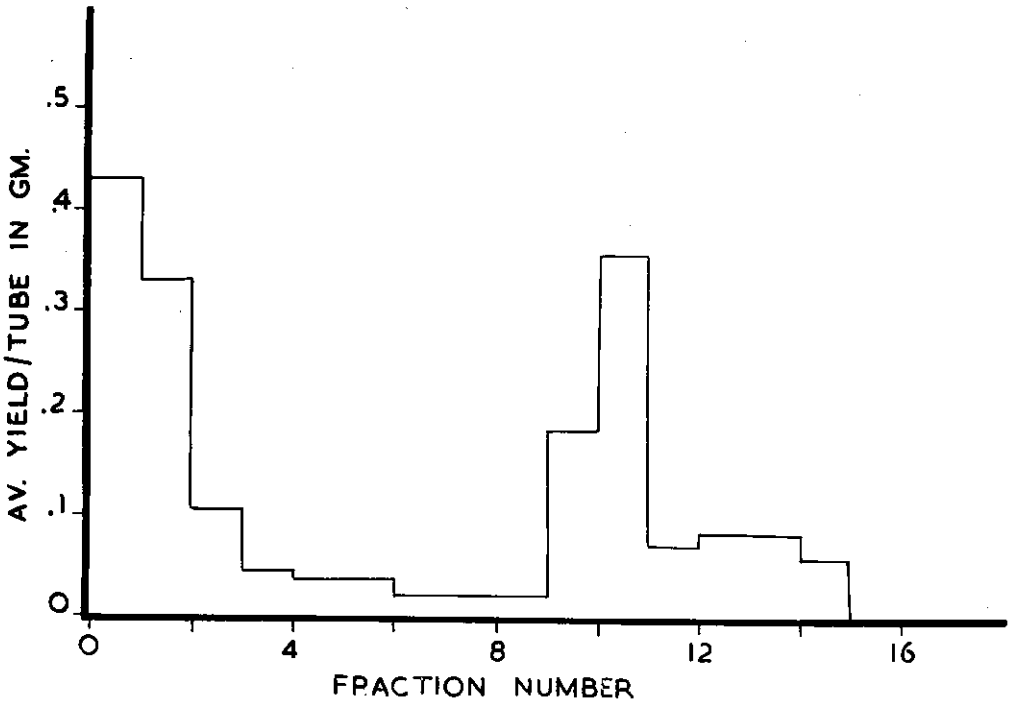

.5
.4
.3
.2
.1
0
AV. YIELD/TUBE IN GM.
0
4
8
12
16
FRACTION NUMBER

FIG. 2B

TABLE 1.

Fraction No.	Yield in G.	Eluent	M.Pr.	Fraction No	Yield in G.	Eluent	M.Pr.
1	0.0200	Pet Et$_2$O (B.P. 40-60°)	116°	16	0.7962	Bz:CHCl$_3$ = 1:1	-
2	0.0196	"	119°	17	0.3978	"	-
3	0.0433	Pet Et$_2$O : Bz = 1:1	120°	18	0.3312	"	-
4	0.1388	Bz	119°	19	0.2386	"	-
5	0.1600	"	125°	20	0.3915	"	-
6	0.1400	"	124°	21	0.4892	Bz:CHCl$_3$ = 1:3	-
7	0.1620	"	116.5°	22	0.1274	"	-
8	0.1146	"	123°	23	0.1696	"	-
9	0.1066	"	118°	24	0.0686	"	-
10	0.0744	"	116°	25	0.0424	"	-
11	0.0600	"	114°	26	0.0196	CHCl$_3$	-
12	0.2306	Bz:CHCl$_3$ = 1:1	-		6.2093		
13	0.2224	"	-				
14	0.4350	"	-				
15	1.0322	"	-				

TABLE 2.

Fraction No.	No. of tubes in fraction	Wt of Fraction	Av. Wt./tube.	Eluent	M.Pt.
1	2	0.8671	0.4336	Bz	122°
2	2	0.6606	0.3303	"	120°
3	3	0.3164	0.1054	"	119°
4	3	0.1274	0.0424	"	-
5	4	0.1502	0.0375	"	-
6	4	0.1316	0.0329	"	-
7	4	0.0710	0.0188	"	103°
8	4	0.0818	0.0204	Bz:$CHCl_3$ = 1:1	103°
9	5	0.1088	0.0218	"	104°
10	5	0.9186	0.1837	"	-
11	3	1.0972	0.0366	"	-
12	4	0.2914	0.0728	"	-
13	5	0.4154	0.0831	Bz:$CHCl_3$ = 1:3	-
14	4	0.3454	0.0863	Bz:$CHCl_3$ = 1:3 "	-
15	7	0.4754	0.0679	"	-

In fractions 1 - 11 some of the oil crystallized and from the m.pt. the crystalline material was Cpd. A. The yield of Cpd. A from fraction 1-11 was 0.3896 g. This was purified by recrystalliztion from Petroleum ether (B.P. 60-80) and 0.344 g. of Cpd. A, mppt. 127° was obtained.

Experiment 9.

100 g. of Acradenia oil were extracted with Girard Rt. P. as in Expt. 8.

From Extract I 73 g. of oil were recovered.

Residue II: Yield 10.399 g. of oil which failed to crystallize. The oil was therefore passed through a chromatograph column to separate the constituents.

Column 3.

10.399 g. of oil from Expt. 9 were dissolved in Benzene and passed through a chromatograph column of diameter 17 mm. containing 300 g. of B. D. H. alumina. The eluate was removed in approx. 50 ml. fractions with a fraction cutter. Details of the column are given in Table 2 and Fig. 2B.

Fractions 1-3 crystallized, yielding 0.693 g. of Cpd. A. The crude product was recrystallized twice from Petroleum Ether (BP 60-80) and 0.656 g. of Cpd. A m.pt. 127° was obtained.

Fraction 7-9 crystallized and 79 mg. of substance of m.pt. 103-104° was obtained. This material was recrystallized from Pet. Ether (B.P. 60-80), and a mixed melting point of the purified substance with Cpd. B, methylalloevodionol, showed no depressions.

Distillation of Acradenia oil

612 g. of Acradenia essential oil was distilled under reduced pressure (P ≈ 0.5 mm) on a 2 ft. distillation column until the temperature at the top of the column reached 60°. The residue of the distillation (99.5 g.) failed to crystallize when seeded with Cpd. A.

Column 4.

35 g. of the residue of the distillation were dissolved in benzene and passed through a chromatograph column, diameter 40mm., containing 1000 g. of B. D. H. alumina. The eluate was withdrawn in 50 ml. fractions by a fraction cutter. Details of the column are given in Table 3 and Fig 4A.

TABLE 3.

Fraction Number	No. of tubes in Frac.	Wt of Frac. in Grams	Av. wt/tube	M.Pt.	Eluent
1	7	0.7452	0.1064	-	Bz
2	4	3.0914	0.7728	-	"
3	4	3.8288	0.9572	-	"
4	4	2.6624	0.6656	134-6°	"
5	6	2.2440	0.3740	134-6°	"
6	8	1.2840	0.1605	-	"
7	10	0.8904	0.0896	-	"
8	10	0.6514	0.0651	-	"
9	10	0.4734	0.0473	-	$Bz:CHCl_3 = 1:1$
10	10	0.6134	0.0613	-	"
11	10	0.8864	0.0886	-	"
12	12	0.8014	0.0668	-	"
13	9	0.7680	0.0853	-	$Bz:CHCl_3 = 1:3$
14	10	1.5394	0.1539	-	"
15	10	2.4068	0.2406	-	$Bz:CHCl_3 = 1:3$
16	13	1.0360	0.0797	-	$CHCl_3$
17	13	0.6034	0.0046	-	"
18	10	2.0499	0.2049	-	$CHCl_3:MeOH =$.
19	10	0.8682	0.0868	-	"
20	10	0.1286	0.0128	-	MeOH.

Fractions 4 and 5 crystallized and yielded a total of 0.119 g. of a yellow subst. of m.pt. 134-136°. This was recrystallized from EtOH twice and boiled with powdered charcoal, and 0.0864 g. of subst. of m.pt. 142.5° was obtained. This was concluded to be substance C.

Experiment 10.

The remainder of the distillation residue (64.5 g.) was extracted with Girard Rt. P.

Reagents: Distillation residue (64.5.g.) Girard Rt. P. (30 g.) dissolved to 420 ml. with Soln a., Na_2CO_3 (28 g.) H_2O (700 ml.) ice (3500)g.) conc. HCl (420 ml.)

Procedure as in Expt. 4. Time of reflux 2 hrs.

From Extract I 50 g. of oil were recovered.

Residue II: Yield 6.143 g. of an oil which failed to crystallize on seeding with Cpd. A or C.

Column 5.

10 g. of Acradenia oil were chromatographed on 300 g. of Lights alumina, in a column of diameter 17 mm. using a large fraction cutter. (50 ml. fraction). The column was terminated after the fraction containing Cpd. A were removed.

Fraction No.	No. of tubes in Fraction	Wt.	Eluent	M.Pt.
1	5	1.2578	Bz	
2	2	1.6069	Bz	114°-116°
3	5	1.0772	Bz	
4	2	0.4352	Bz	

Fraction 2 crystallized, and yielded 0.288 g. of Cpd. A. Although fractions 1 and 3 were seeded with Cpd. A, they did not crystallize. Yield of Cpd. A = 2.88%

Column 6.

30 g. of Acradenia essential oil were chromatographed on 1000 g. of Light's alumina in a column of diameter 4 cm. using a large fraction cutter (50 ml.) For details of the column see Table 4 and Fig. 3A.

Fractions 2 and 3 yielded a total of 0.9103 gm. of crude Cpd. A, m.pt. 112-115°.

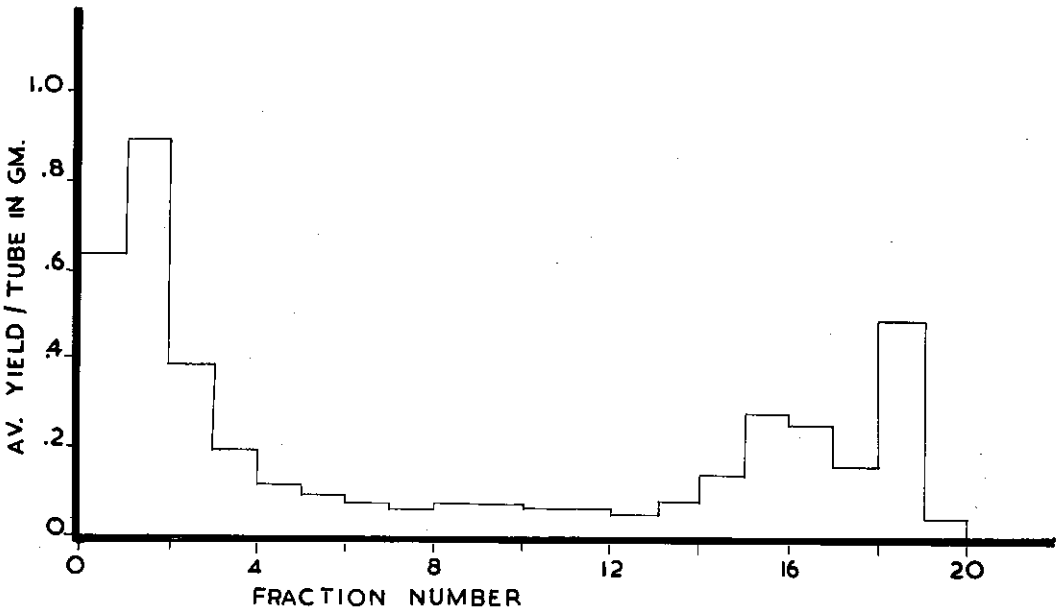

AV. YIELD / TUBE IN GM.
1.0
.8
.6
.4
.2
0
0
4
8
12
16
20
FRACTION NUMBER

FIG. 3A

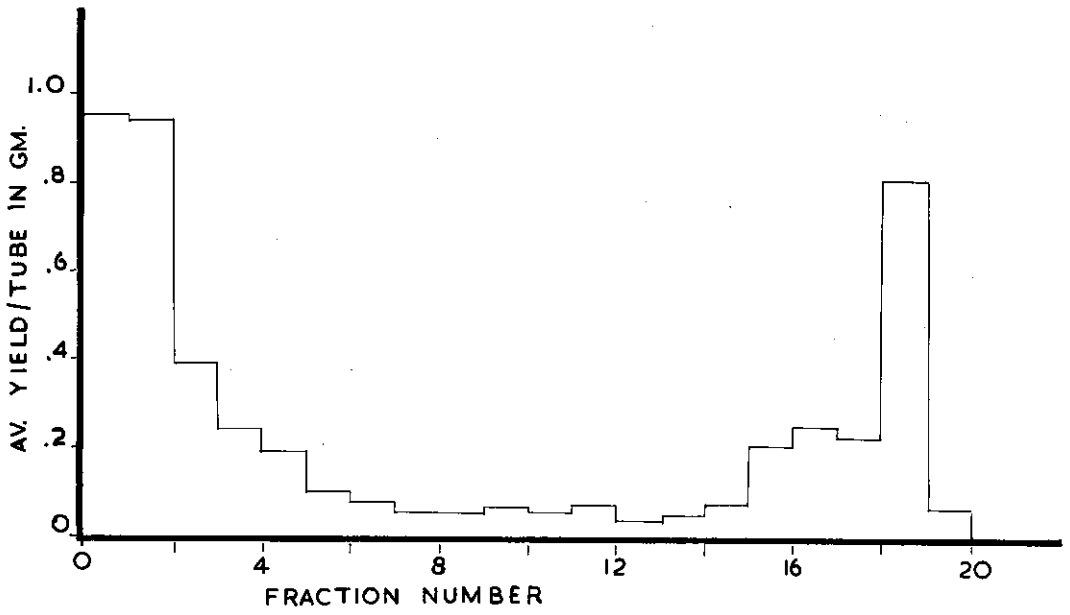

AV. YIELD/TUBE IN GM.
1.0
.8
.6
.4
.2
0
0
4
8
12
16
20
FRACTION NUMBER

FIG 3B

TABLE 4.

FRACTION NUMBER	NO OF TUBES IN FRAC.	FRAC. NO. IN FIG. 3A.	WT OF FRAC. IN GRAMS.	AV. WT/TUBE	M.PT	ELUENT
1	5	1	3.2176	0.6435	-	Bz
2	5	2	4.4958	0.8992	112-115°	"
3	5	3	1.9314	0.3863	112-115°	"
4	5	4	0.9876	0.1975	-	"
5	5	5	0.6458	0.1292	-	"
6 a	5	6	0.4674	0.0935	-	Bz:$CHCl_3$ = 3:2
6 b.	5	7	0.3500	0.0700	-	"
7 a.	5	8	0.2986	0.0597	-	Bz:$CHCl_3$ = 1:1
7 b.	5	9	0.3276	0.0655	-	"
8 a.	5	10	0.3381	0.0676	-	Bz:$CHCl_3$ = 1:3
8 b.	5	11	0.2961	0.0592	-	"
8 c.	5	12	0.2964	0.0593	-	"
8 d.	5	13	0.2766	0.0353	-	"
9 a.	5	14	0.3930	0.0786	-	$CHCl_3$
9 b.	5	15	0.7331	0.1466	-	"
10 a	5	16	1.3676	0.2735	-	$CHCl_3$:MeOH = 3:1
10 b.	5	17	1.2861	0.2572	-	"
10 c.	5	18	0.7866	0.1573	-	"
11 a	5	19	2.5064	0.5013	-	$CHCl_3$:MeOH = 3:2
11 b.	5	20	0.2306	0.0461	-	"
			21.2324			

TABLE 5.

FRACTION NUMBER	NO. OF TUBES IN FRACTION	FRAC. NO. IN FIG. 8B.	WT. OF FRAC. IN GMS.	AV. WT./TUBE	M. PT.	ELUENT
1	5	1	4.8171	0.9634		Bz
2	5	2	4.7768	0.9554	112-114	"
3	5	3	2.0015	0.4003	112-114	"
4 a	5	4	1.2562	0.2512		"
4 b	5	5	0.9646	0.1929		"
5 a	5	6	0.5772	0.1154		Bz:$CHCl_3$=3:2
5 b	5	7	0.4032	0.0806		"
5 c	5	8	0.3307	0.0661		"
6 a	5	9	0.3297	0.0659		Bz:$CHCl_3$=1:1
6 b	5	10	0.3600	0.0720		"
6 c	5	11	0.3022	0.0604		"
7 a	5	12	0.3477	0.0695		Bz:$CHCl_3$=3:1
7 b.	5	13	0.1890	0.0378		"
8 a	5	14	0.2616	0.0522		$CHCl_3$
8 b	5	15	0.3758	0.0752		"
9 a	5	16	1.0760	0.2152		$CHCl_3$:MeOH=.
9 b	5	17	1.2722	0.2544		"
9 c	5	18	1.1850	0.2370		"
10 a	5	19	4.0782	0.8156		$CHCl_3$:MeOH:
10 b.	5	20	0.3532.	0.0706.		"

Column 7.

Repetition of Column 6. For details see Table 5 and Fig. 3B. Fractions 2 and 3 crystallized on seeding, and yielded a total of 1.034 g. of crude Cpd. A m.pt. 112-114°.

The crude Cpd. A from Columns 6 and 7 was combined and recrystallized from Petroleum Ether (B.P. 60-80). Three recrystallizations failed to raise the m.pt. above the range 116-120° It seemed that an additional substance was present and so the products and mother liquors from the recrystallization were chromatographed. (Column 8)

Column 8. Separation of Cpds. A and C. from columns 5, 6, and 7

2.257 g. of crude Cpd. A were dissolved in Pet. Ether (BP 40-60) and chromatographed on 50 g. of Lights' alumina, using a small fraction cutter (20 ml. fractions). The diameter of the column used was 15 mm. For details of the Column see Table 5 and Fig 4B.

All fractions crystallized; from the melting pts, Fractions 1 and 2 containd Cpd. A, and 5 and 6 Cpd. C.

Fractions 3 and 4 contained a mixture of Cpds. A and C. As the crystals were well developed and easily distinguishable as A is white and C is yellow, a rough separation of these two fractions into Cpds. A and C was made with the aid of a hand lens.

A total of 1.959 g. of Cpd. A and 0101 of Cpd. C was obtained from this column.

The Cpd. A from fractions 2, 3, and 4 was recrystallized twice from Petroleum Ether (BP. 60-80°)

FIG. 4B

FRACTION NUMBER
0
4
8
12
16
YIELD IN GM.
0
.1
.2
.3
.4

FIG. 4A

FRACTION NUMBER
0
4
8
12
16
AV. YIELD/TUBE IN GM.
0
.2
.4
.6
.8
1.0

TABLE 6.

FRACTION NUMBER	WT OF FRAC IN G.M.	M. Pt	ELUENT	FRAC. NO IN FIG. 43.	FRACTION NUMBER	WT OF FRAC IN G.M.	M. Pt.	ELUENT	NO OF TUBES IN FRAC.
1a	0.3721	114-6°	Pet Et$_2$O : Bz = 9:1	1	5	0.0305	140°	Bz : CH_2Cl_2 = 1:1	5
1b	0.3833			2	6.	0.0367	140°	"	4.
1c	0.4190			3					
2a	0.2008	114-118°	"	4					
2b	0.1924			5					
2c	0.1330			6					
3a	0.1136	113-117°	"	7					
3b	0.0138			8					
3c	0.0414			9					
4a	0.0318	115-120°	Bz.	10					
4b	0.0304			11					
4c	0.0320			12					
4d.	0.0468			13					

No. of tubes in Fracs 1–4 = 1.

SECTION III

Investigation of Compound A.

1. Summary of known information.

Dr. Komzak commenced work on compound A and the following is a brief summary of the information which he ascertained. On analysis the molecular formula was found to be $C_{19}H_{22}O_4$.

	C	H	MeO	M.W.
Calc. for $C_{19}H_{22}O_4$	72.6	7.0	9.9	314
Fd. by C.S.I.R.O.	72.8	7.25	9.9	
Fd. Komzak	72.92	7.35	10.93	312
			10.43	305

On analysis C-Me fd. = 10.9%. 1 C Me calc. = 4.70% ∴ approx. 2.25 C-Me grps.
From these figures the molecule has one methoxy group.

Compound A is a carbonyl compound, since it yields a dinitrophenylhydrazone, m.pt. 183-4°, with formula $C_{25}H_{26}O_7N_4$ (Fd.: C, 60.82; H, 5.26; N, 11.3. Calc. for $C_{25}H_{26}O_7N_4$: C, 60.7; H, 5.26; N 11.3.) Thus there is one carbonyl group present as a ketone since A will not react with sodium bisulfite. A will not give an iodofrom test. Compound A is unsaturated. With bromine in carbon tetrachloride it yields a dibromo addition product, m.pt. 141-3°. (Fd.: (1) C, 46.93; H, 4.10; Br 35.9. (2) C, 47.12; H, 4.09; Br, 35.7. Calc. for $C_{19}H_{22}O_4Br_2$: C, 48.0; H, 4.64; Br, 33.7.)

Hydrogenation of A in ethanol with PtO_2 catalyst yields a crystalline product m.pt. 143°. From the analysis it is not quite clear whether it is a dihydro or tetrahydro derivative.

	C	H	O
Found.	71.97	8.18	20.2
Calc. for $C_{19}H_{24}O_4$	72.13	7.66	20.22
Calc. for $C_{19}H_{26}O_4$	71.70	8.18	20.13.

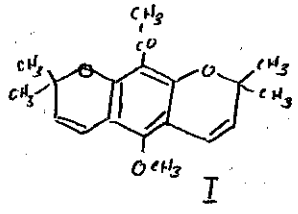
with $FeCl_3$ A gives no reaction therefore there are no phenolic hydroxy groups.

On boiling with 25% KOH in an attempt at saponification, the substance slowly distilled over unchanged into the condenser, showing its steam volatility.

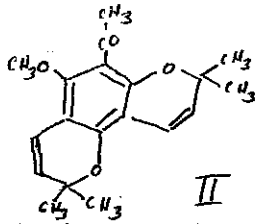
With conc. H_2SO_4 A gives a bright cherry red coloration.

2. Suggested Structure of A.

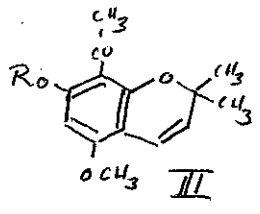
Two of the four oxygen atoms in A are accounted, for and since A is not acidic, will not react with $FeCl_3$, and cannot be saponified, the other two are assumed to be present in ether linkages. In view of its occurrence with B(R=Me) Methyl allo evodionol, a 2.2 dimethyl chromene ring structure has been suggested. This could have either a linear (I) or angular (II) form:



I



II



III

Compound B contains a phloroglucinol nucleus, and Compound C has been shown to contain one also, and it seems reasonable to assume that A is most probably based on a phloroglucinol nucleus.

The fact that A will not give an iodofrom test is not conclusive evidence that no methyl ketone group is present. In the closely related allo evodionol series (III,R=H,Me) the acetyl group is significantly hindered, and will not give an iodoform test (7). There is some evidence from spot tests carried out on A that there is a methyl ketone group, since it gives a positive sodium nitroprusside test. Methyl allo evodionol will not yield a dinitrophenylhydrazone (8 9), and since structure II for A contains the same groupings adjacent to the carbonyl group as methyl allo evodionol, it seems that I is a more probable structure.

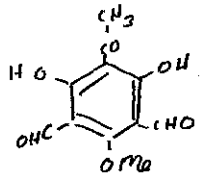
The C-Me analysis for A does not correspond to an integral number of groups, and neither I nor II would be expected to give an integral number. The degree of unsaturation of A is not clear from the available evidence. The addition compound formed with bromine is a dibromo compound but it is not clear whether the hydrogenated A is a di or tetrahydro compound.

It was hoped to derive further information about A from oxidation with K MnO_4 in acetone solution. By analogy with other chromene structures, on oxidation with K MnO_4 A would be expected to give a di- or tetra- carboxylic acid depending on whether ring fission occurred in one or both chromene rings at the double bond (9).

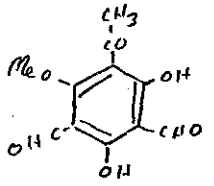
In a small scale oxidation Konzak obtained a small amount of a white crystalline material, insufficient for any identification to be made, and it was hoped to obtain some more of this compound.

Therefore 4 gm. of A were oxidized with K MnO_4 in acetone, but on working up the various fractions no crystalline products were obtained. Some of the fractions were oxidized further in the hope of obtaining an identifiable product, but further oxidation yielded only a minute quantity of a white amorphous substance, and so no information could be derived from this work. The oxidation could not be repeated as there was insufficient compound A available.

Further work on compound A is necessary for the confirmation of its structure. Ozonolysis would show whether the structure is based on phloroglucinol. By analogy with the general pattern of ozonolysis experiments with 2.2 dimethyl chromens (9), ozonolysis of I should yield IV, and II V.

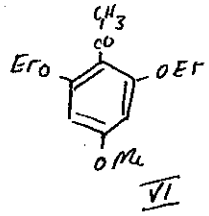


IV

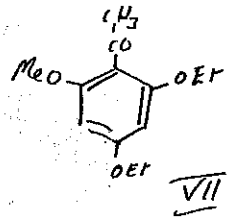


V

On subsequent esterification and removal of the CHO groups by oxidation and decarboxylation, IV would give VI and V, VII.



VI



VII

This would also show position of attachment of the rings if the ethyl esters were used.

Experimental

Spot Test for Methyl Ketones. (10, p. 160)

One drop of alcoholic test solution was mixed in a porcelain crucible with one drop of a 5% solution of sodium nitroprusside and one drop of 30% NaOH soln. After a short time 1 - 2 drops of glacial acetic acid were added. A red to blue colouration indicates a methyl ketone.

This test was carried out on compound A, on acetone as a test compound, and a blank using alcohol only was carried out also.

Substance	Initial Colour	Colour on addn. AcOH.
Blank	bright yellow	no change
Cpd. A	bright yellow	bluish purple
Acetone	Bright yellow	crimson changing to purple.

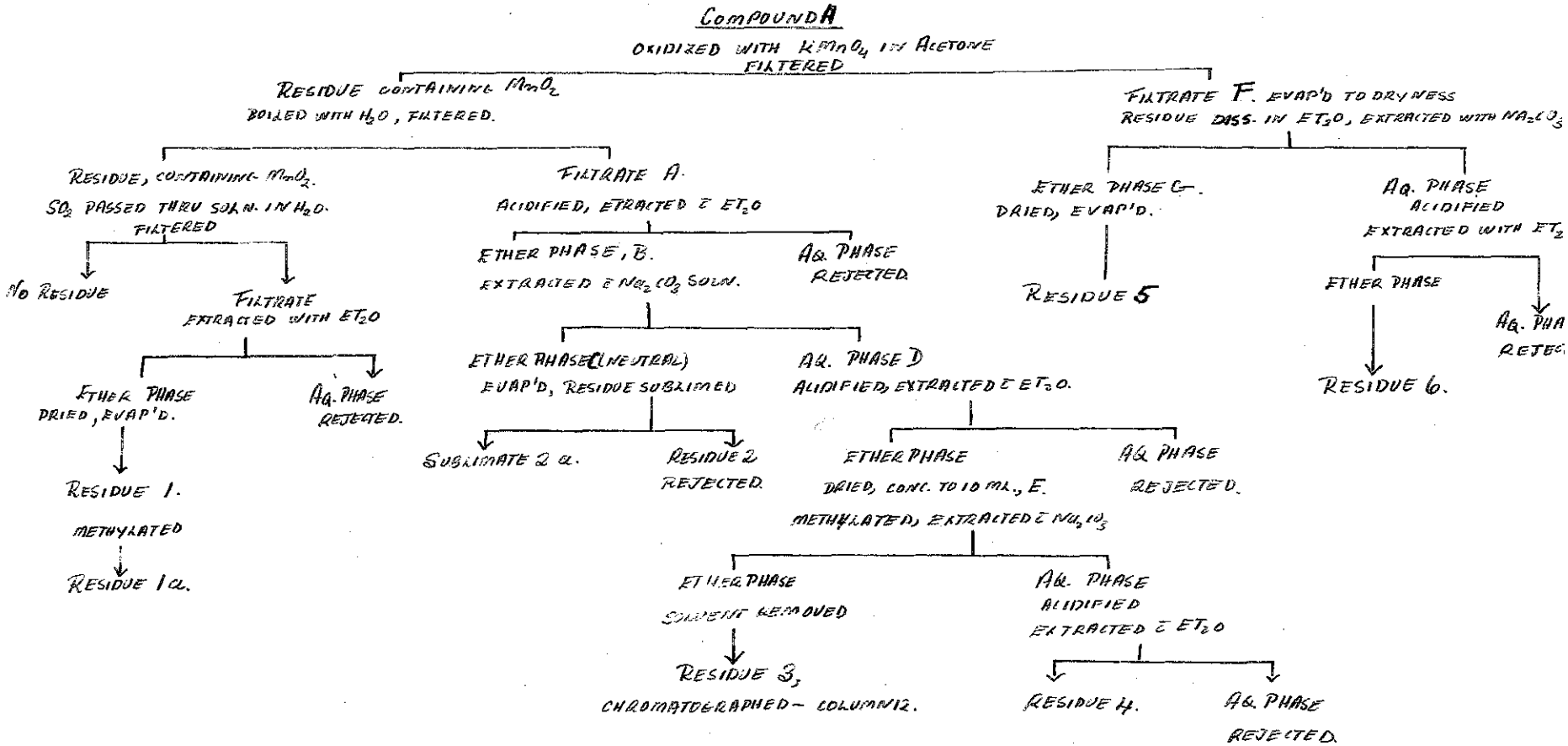
Oxidation of compound A.

The procedure used for oxidation was that of Lahey (11). The acetone used for the oxidation was first shaken with K MnO_4, distilled and dried over anhydrous sodium sulphate. 4 gm. finely powdered compound A were placed in a beaker with 150 ml. dry acetone. Finely ground K MnO_4 was added and the mixture stirred mechanically. Samples were withdrawn at intervals and centrifuged in a muro centrifuge, and the oxidation was considered complete when the solution remained pink after ten minutes with no further addition of K MnO_4. A total of 6.6 gm. K MnO_4 were consumed in the oxidation.

The oxidation solution was worked up as shown in fig. 5.

Summary of Oxidation of Cpd. A.

FIG. 5.



The acetone solution from the oxidation was filtered and the residue containing MnO_2 boiled with water to extract any substances adsorbed by the MnO_2, the solution was filtered and the MnO_2 residue treated with SO_2 to render the MnO_2 soluble. The solution thus formed was extracted with ether to remove organic compounds, and the ether phase dried with anhydrous sodium sulphate and the solvent removed by evaporation.

Residue I = 0.3378 gm., was methylated with diazo methane but the product failed to crystallize, and so was subjected to further oxidation. The filtrate A was acidified and extracted with ether in a 500 ml. liquid extractor for 48 hrs., and the ether solution B on drying and evaporation yielded 2.645 g. of a liquid which failed to crystallize and so was redissolved in ether and extracted with Na_2CO_3 soln. to separate the neutral and acid fractions.

Ether solution C, the neutral portion, on drying and evaporation yielded 0.3283 g. of a liquid containing a whitish material. The liquid was sublimed using a Towers Widnes High Vacuum pumping unit at a pressure of 10^{-5} mm. and a temperature of 90°. A minute yield of a white sublimate was obtained, insufficient for identification purposes, and was reoxidized at a later stage. The residue, a black oil, had to be rejected.

The aqueous phase D, containing the acid fraction, was acidified and extracted with ether in a 500 ml. liquid liquid extractor for 48 hours. The ether phase was dried, methylated with diazo methane, and extracted with Na_2CO_3 soln. to remove any acid substances remaining.

Residue III, (0.267 g.) which was neutral, presumably containing methyl esters was chromatographed on a column of diameter 10 mm., using 8 g. B.D.H. Al_2O_3 - Column 12.

Fraction Number	Wt.(in grams)	Eluent
1	0.0935	Bz:Pet.Et_2O(40-60°)1:2
2	0.0550	Bz:Pet.Et_2O(40-60°)1:1
3	0.1025	$CHCl_3$: Bz: 1:1
4	0.0320	$CHCl_3$: Bz: 1:1
	0.2630	

None of the fractions crystallized.

Residue IV = 1.148 gm.

Filtrate F was evaporated to dryness, and the residue dissolved in ether and extracted with Na_2CO_3 soln. The ether phase on drying and evaporation yielded Residue 5, 0.0213 gm. of crystalline substance, m.pt. 127°, identical with compound A. Thus the oxidation was not complete.

Residue 6 was 0.2905 gm. of a liquid which failed to crystallize.

Fractions 1-4 of Column 12, Residues 1a, 2a and 6 a total of 0.6139 were oxidized with powdered $KMnO_4$ in 50 cc. acetone and treated in a similar manner to Oxidation I. See fig 6.

The oxidation product, 0.2835 gm. was a liquid containing a white amorphous substance which failed to crystallize. It was taken up in benzene and chromatographed on 6 gm. B.D.H. alumina in a column of diameter 10 mm., Column 13.

Fraction No.	Yield (in grams)	Eluent
1	0.1106	Bz
2	0.0874	Bz:$CHCl_3$ 1:1
3	0.0410	$CHCl_3$
	0.2390	

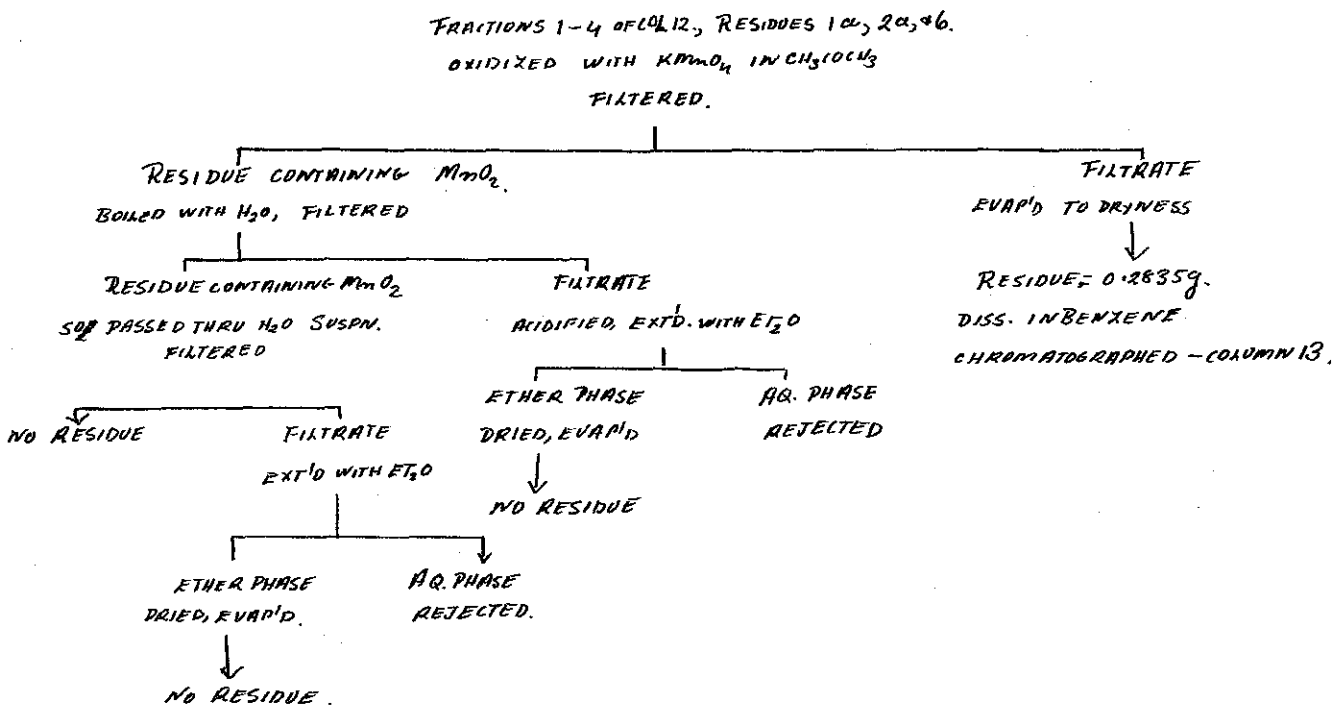
The fractions all failed to crystallize, although they were dissolved in EtOH, filtered and cooled.

Preparation of Diazo Methane

N Methyl N'nitroguanidine (12)

A solution of 52 g. of nitroguanidine in 150 ml. of water containing 60 g. KOH was prepared by heating the mixture to 40°, and methylamine hydrochloride (67.5 g.) was added with stirring. A viscous sludge formed and NH_3 was evolved. The temperature was increased steadily to 59° over a period of 8 minutes and maintained at 59-61° for 23 minutes, the reaction mixture being stirred continuously. At the end of this time the clear solution was cooled in an ice-water bath to 6°. A white ppt. formed which was filtered off, washed with 150 ml. cold water and dried in an oven at 60°. The crude product melted at 150-154°

FIG. 6.


FRACTIONS 1-4 OF COL 12, RESIDUES 1a, 2a, & 6.
OXIDIZED WITH $KMnO_4$ IN CH_3COCH_3
FILTERED.
RESIDUE CONTAINING MnO_2.
BOILED WITH H_2O, FILTERED
FILTRATE
EVAP'D TO DRYNESS
RESIDUE = 0.2835g.
DISS. IN BENZENE
CHROMATOGRAPHED - COLUMN 13.
RESIDUE CONTAINING MnO_2
SOL PASSED THRU H_2O SUSPN.
FILTERED
FILTRATE
ACIDIFIED, EXT'D. WITH Et_2O
NO RESIDUE
FILTRATE
EXT'D WITH Et_2O
ETHER PHASE
DRIED, EVAP'D
AQ. PHASE
REJECTED
NO RESIDUE
ETHER PHASE
DRIED, EVAP'D.
AQ. PHASE
REJECTED.
NO RESIDUE.

and the yield was 25 g. (43%) It was recrystallized once from water (3 ml./g.) to remove inorganic material and a second time from 95% EtOH (8 ml/g.) and 18 g. of m.pt 155° were obtained.

N-methyl N-nitroso N'-nitroguanidine (13)

N methyl N' nitroguanidine (18 g.) was dissolved in 180 ml. H_2O by the addition of 54 ml. HNO_3. The soln. was cooled in an ice-water bath and $NaNO_2$ (22.3 g.) dissolved in 36 ml. H_2O was added over a period of five minutes. The reaction mixture was stirred mechanically during this process and afterwards for a period of twenty minutes. The yellow crystalline product was filtered and washed with cold water. The crude product melted at 112-113°. The yield was 6.0 g. (30%).

Diazo-Methane Solution (13, 14, 15)

In a 250 ml. r.b. flask were placed N methyl-N nitroso N'-nitroguanidine (6 g.), ether (130 ml.) and 50% KOH soln (20 ml.). The flask was fitted with a condenser set for downward distillation To the lower end of the condenser was attached an adapter passing through a two-holed rubber stopper and dipping below the surface of 40 ml. of pure ether in a 300 ml. concial flask. The exit gases were passed through a second 40 ml. portion of ether in a 300 ml. concial flask. Both conical flasks were cooled below 0° in an ice-salt bath.

The reaction flask was placed on a water bath at 50° and shaken occasionally. The ether, containing diazomethane was distilled until it passed over colourless. About $\frac{2}{3}$ of the ether solution was distilled off. It is dangerous to distil all the ether off as an explosion may occur. The ether solutions in the receivers were combined and used for esterification.

Esterification with Diazomethane (15)

Residue 1 was dissolved to 10 ml. with ether and the ether solution of diazomethane was added. N_2 was evolved and the yellow colour of the diazomethane solution disappeared. Diazomethane solution was added till the solution remained yellow after standing overnight, and then the excess diazomethane was removed by warming on a water bath.

Ether solution E was methylated in a similar manner.

SECTION IV

Investigation of Compound C.

Compound C is a pale yellow crystalline compound, m.pt. 143°, which was first isolated from the essential oil Acradenia Franklinii by Dr. A. R. Komzak. He obtained small amounts of C from chromatography of the oil, and from distillation of the oil under vacuum. Compound C crystallized out from the fraction with a boiling range of 100°-130°C when the oil was distilled at a pressure of 1 mm. of mercury. The molecular formula of C is $C_{11}H_{14}O_4$ (Fd.: C, 62.92; H, 6.64; O, 30.00; M.W., 215; MeO, 39.46; OMe, 13.9. Calc. for $C_{11}H_{14}O_4$: C, 62.8; H, 6.70; O, 30.43; M.W., 210.21)

C is a carbonyl compound since it forms a 2,4, dinitrophenylhydrazone, which crystallized from Ethyl acetate in bright red needles, m.pt. 275°. (Fd: C, 52.22; H, 4.91; N, 14.29 The investigation of Compound C was taken over at this stage.

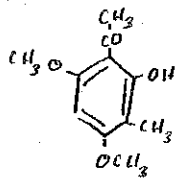
Structure of Compound C.

From this information it can be seen that there are two methoxy groups and one carbon methyl group in the molecule. (Fd.: MeO, 29.46; C-Me, 12.8. Calc. for $C_{11}H_{14}O_4$: 1 MeO, 14.77; 1 C-Me, 12.8.)

Cpd. C gives a purple brown ferric chloride test in alcoholic solution indicating the presence of a phenolic hydroxy group. The colouration in the ferric chloride test is unaltered by the addition of water. Cpd. C gives a positive iodoform test, which shows that the carbonyl group is present as a methyl ketone. Thus all four oxygen atoms are accounted for, and only a CH_2 group is unaccounted for. This must be present as a methyl substituent on the benzene ring. Hence the structure of C must be a benzene nucleus with two methoxy groups, a hydroxy, a methyl, and a methyl ketone group substituted in it.

2-hydroxy, 4, 6 dimethoxy, 3 methyl Acetophenone.

On searching the literature an account was found of the compound 2-hydroxy, 4,6, dimethoxy, 3 methyl acetophenone (I), which was prepared by Curd and Robertson (16) in 1933. It is a colourless crystalline compound, insoluble in water, with m.pt. 141-143°C, depending on the method of preparation. It gives a purple brown ferric chloride test in alcoholic solution which is unchanged by the addition of water. The only derivative prepared was an acetate,



I

Synthesis of 2-OH, 4,6 di-MeO, 3 Me Acetophenone

As it seemed probable from the available information that C was identical with 2-OH, 4,6 di-MeO, 3 Me Acetophenone (I), the latter was synthesized.

Curd and Robertson synthesized I in three ways:-

1. By condensation of Methyl phloroglucinol α-di methyl ether with acetonitrile.
2. By methylation of phloroacetophenone with K_2CO_3 and Me I.
3. By methylation of methyl phloroacetopheneone with K_2CO_3 and Me I.

The second method of preparation was chosen as it was straight forward and had the simplest starting material. A pale yellow crystalline product was obtained. m.pt. 142-143°. The product of Curd and Robertson's syntheses was colourless, but no amount of purification seemed to remove all traces of colour either from the synthetic material or from the naturally occurring substance. Both remained pale yellow.

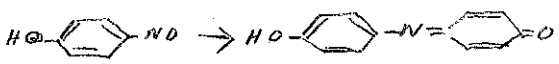
Proof of Identity of C and I.

1. A mixed melting point determination was carried out, and there was no depression. This, however, is not an absolute criteria of identity.

m.pt. C	mixed m.pt.	m.pt. I.
142-143°	142-143°	142-143°

2. As confirmatory evidence the dinitrophenylhydrazone of I was prepared. The dinitrophenylhydrazone crystallized from ethyl acetate in bright red needles, m.pt. 163°.
Analysis. [Fd:(i): N, 14.22; C 53.60; H, 4.90; (ii) N, 13.75, C, 53.49, H, 5.19%
Calc. for $C_{17}H_{18}O_7N_4$: N, 14.36, C, 52.3, H, 14.36.]
The dinitrophenylhydrazone prepared by Dr. Komzak from C, had m.pt. 275°. It was thought the two might be geometrical isomers, and so a saturated solution was made of the D.N.P. of I. and seeded with a crystal of the D.N.P. of C, but this had no effect as the product obtained still melted at 163°. This was repeated several times with the same result.

3. A number of short tests were made on compounds I and C. All the tests applied were based on the fact that phenols and phenol ethers with vacant para positions form paranitroso derivatives with nitrous acid which give indophenols by condensation with excess phenol in the presence of sulphuric acid,



Two of the tests gave no action with either compound and the third gave the same series of colour changes in both. This is not conclusive evidence for their identity, or non-identity.

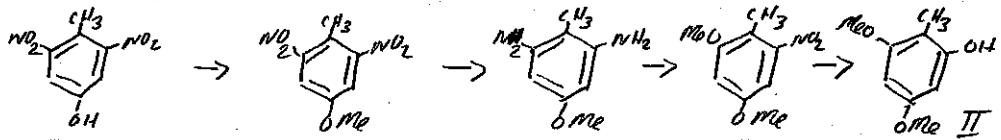
4. A small paper chromatogram of solutions of the two compounds was run, but development failed to give any spots.

5. As a final test of the identity of the compounds, purified samples were sent of the University of Technology to have their infra red spectra taken.

5 mg. of C which was all the material available, and 15 mg. of 2-OH, 4,6, diMeO, 3 Me phloroacetophenone were sent. On the material the spectra of the two compounds ~~was~~ were shown to be identical. The spectra of both showed certain interesting features. The carbonyl frequency in both appeared to be lowered and there was no evidence of a hydroxy group being present. There was no evidence either of the shift in the band due to the OH group which can occur

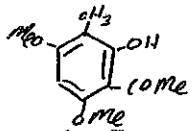
if a carbonyl and adjacent OH group are linked through a hydrogen band. Further synthetic substance has been sent so that the spectra in solution can be taken. There is no doubt about the structure of 2 OH, 4,6diMeO, 3 Me acetophenone, as it was conclusively prove by the work of Curd and Robertson (16).

They first conclusively proved the structure of methyl phloroglucinol α-dimethyl ether (II) by synthesizing it from 2, 6 dinitro paratoluidine through stages:



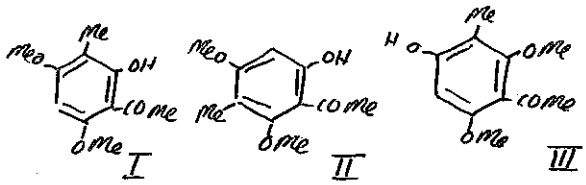
II

It was found that treatment of phloroacetophenone with MeI and K_2CO_3 in boiling dietone resulted in nuclear dohylation, and the formation of a C-methyl phloroacetophenone (I)



I

The orientation of groups in I was shown as follows:
The same compound is also obtained by the condensation of methylphloroglucinol α-di methyl ether and acetonitrite by the Hoesch procedure, and thus the OH group is ortho to the C-Me group and so of the three possible formulae I, II, III, II is excluded.



I II III

The intense ferric chloride reaction given by the compound indicated that the OH was also ortho to the COMe group, thus excluding III. In any case, a compound of the latter type if formed in the reaction mixture would be expected to undergo further alkylation and yield a trimethyl ether. That the hydroxy is ortho to the carbonyl group is confirmed by the fact that on condensation with anisaldehyde the compound gave the chalkone IV which on ring closure gave the flavanone V.

Conclusion:

The structure of compound C is thus established as being identical with 2-OH, 4, 6 di MeO 3 Me acetophenone. There appears to be no previous record of this compound occurring naturally. The extent to which 2 OH, 4, 6 di MeO 3 Me acetophenone occurs in Acradenia Franklinii is not known, as the yields so far obtained cannot be regarded as quantitative, but from the yield of Column 8, it may be estimated as 0.168% as a minimum value.

Experimental

1. Group tests.

The group tests on compound C were conducted by the methods given in Shriner and Fusen(1).

$FeCl_3$ test: purple brown in alcoholic solution, unchanged by addition of water (1 p. 98)

Iodoform test: white precipitates (1, p.138)

A blank test and one on acetophenone as a type compound were conducted simultaneously to check the result.

2. Synthesis of 2 OH, 4, 6 di MeO, 3 Me phloroacetophenone.

Preparation of Phloroacetophenone (17, 18)

In a 300 ml. bolt neck flask fitted with a rubber stopper and an inverted thistle funnel were placed 25.2 gm. phloroglucinol which had been dried by heating for 12 hours at 120° 16.4 gm. acetonitrile which had been dried over $CaSO_4$, 5 gm. finely powdered $ZnCl_2$ and 100 ml. sodium dry ether. The inverted thistle funnel is to provide a wide necked entry tube to prevent clogging owing to the separation of solid ketimine hydrochloride. The whole was cooled in an ice-salt mixture and dry hydrogen chloride prepared from concentrated H_2SO_4 and HCl (18, p. 176) was passed rapidly through the solution for 2 hours, with occasional shaking. A bulky orange precipitate of ketimine hydrochloride formed during this stage. The flask was placed in the refrigerator for 24 hours and the reaction mixture solidified. This prevented the passing of HCl gas through the mixture for an additional 2 hours as recommended. The solid was transferred with approx 1 l. hot water

to a 3 litre r.b. flask fitted with a reflux condenser, and boiled vigorously for 2 hours over a wire gauze. The mixture was cooled and approx 4 gm. powdered decolorizing carbon were added. The solution was boiled for 5 minutes, and the hot solution filtered with suction through a preheated Büchner funnel. The decolorizing carbon was extracted twice with 100 ml. portions of boiling water and the filtrate added to the main filtrate. This was left to stand overnight, and the pale yellow crystals which formed were filtered off and dried in an oven at 120° to remove water of crystallization.

The yield was 11.40 gm. m.pt. 212°.

The yeild was 33% since theoretical yield is 29 gm.

The low yeild was probably due to the solidification of the reaction mass at an early stage in the preparation. In case the ketoxime was incompletely hydrolysed the solution was boiled for an additional hour, and cooled, but no further yield was obtained.

The crude product was recrystallized in hot H_2O (37 ml/gm.) and 9.68 gm. of phloroacetophenone m.pt. 217° were obtained.

Methylation of Phloroacetophenone (16)

6 gm. phloroaceotphenone, 30 cc. MeI, and 18 gm K_2CO_3 were refluxed with 60 cc. acetone in a 250 ml. r.b. flask for 3 hours. The acetone solution was filtered to remove K_2CO_3 and evaporated to dryness. The residue was taken up in ether, the KI formed during the reaction remaining undissolved.

The ether solution was washed x 4 with Na_2CO_3 to remove unchanged phloroacetophenone, and then washed x 4 with 10% NaOH solution to remove the 2 OH, 4, 6 di MeO, 3 Me phloroacetophenone. On acidification with H_2SO_4 the Na_2CO_3 extract yielded a small amount of phloroacetophenone. The NaOH extract on acidification yielded 1.57 gm. of a mixed product of white and yellow crystals which was separated by chromatography. (See Chromatography Columns 9 and 10). On evaporation the ether solution yielded 0.986 gm. yellow crystalline material m.pt. 142°. This was concluded to be 2 OH, 4, 6 di MeO, 3 Me phloroacetophenone (I) Recrystallization from EtOH yielded 0.846 gm. I m.pt. 143°

Separation of Product of Synthesis by Chromatography: Column 9.

A small amount of the mixed product from the Synthesis was chromatographed first to see whether the method proved satisfactory.

0.4135 gm. of the mixed product were dissolved in a mixture of 1 part benzene to 3 parts of Petroleum Ether (B.P. 40-60°) and chromatographed on 23 gm. B.D.H. alumina. The column was made up in a mixture of benzene: Petroleum Ether (40-60) = 1:3.

Fraction No.	No. of tubes in fraction	Wt. of fraction in gm.	Eluent	M.Pt
1	5	.0940	Bz:PetEt_2O 1:3	132°
2	10	.1844	Bz:PetEt_2O 1:3	130-135°
3	8	.0564	Bz:PetEt_2O 1:1	128°
4	20	.0307	Bz	75-76°
5	35	.0246	Bz:$CHCl_3$ 1:1	75-76°
6	20	.0103	Bz:$CHCl_3$ 1:1	75-76°
		.4004		

All fraction crystallized, fractions 1-3 in yellow crystals and 4-6 in white ones.

Column 10

Since the method of chromatography was suitable for separation the remaining 1.1577 gm. were now chromatographed on 35 gm. B.D.H. alumina, using the small fraction cutter.

Fraction No.	No. of tubes in fraction	Wt. of fraction in gm.	Eluent	M.Pt.
1	30	.3294	Bz:PetEt_2O 1:3	136°
2	30	.0442	Bz:PetEt_2O 1:3	133-5°
3	20	.1132	Bz:PetEt_2O 3:1	133-5°
4	15	.0424	Bz	74-76°
5	10	.1733	Bz: $CHCl_3$ 1:1	75-77°

This column was not completed owing to an accident but the fractions containing I had already been obtained so this was not serious.

Fractions 9_1 to 9_3 and 10, to 10_3 were combined, dissolved in EtOH, and boiled with decolorizing powdered carbon and then filtered twice to remove the carbon. The solution was then cooled, a little water added, and the 2 OH, 4,6 di MeO, 3Me acetophenone which crystallized

out had m.pt 139°. Two recrystallizations from EtOH raised the melting point to 143° but the crystals were still a pale yellow. The material obtained by Curd and Robertson was colourless, and so a further attempt was made to remove the coloration by dissolving the material in MeOH, boiling it with filter acid, filtering it, and cooling to let the material crystallize. The crystals remained a pale yellow, and the melting point was not raised. From columns 9 and 10 a total of 0.8216 gm. crude 2OH, 4,6 di MeO_3 Me acetophenone was obtained. The white crystalline product also obtained from the chromatography was recrystallized twice from EtOH and the m.pt. was raised to 85°. This was probably 2, 4 di methyl ether of phloroacetophenone, m.pt. 86-8°, formed by incomplete methylation. Curd and Robertson found no second product when they methylated phloroacetophenone. The total yield of 2 OH 4, 6 di MeO_3 Me phloroacetophenone from the synthesis was 0.8216 + 0.9863 = 1.8079 gm. (48% yield).

3. *Spot* tests on compound C and 2 OH 4, 6 di MeO_3Me acetophenone (19)

(a) 0.05 - .1 gm. of substance to be tested was placed in a test tube with one drop of freshly distilled aniline 5 ml water and 2 ml. of a suspension of bleaching powder. This was applied to C, I and a blank, and the colour changes in all three were indistinguishable. The solutions turned a dirty mauve which changed to indigo blue on the addition of ammonium hydroxide.

(b) Liebermann's reaction (10, p.132)

A drop of test solution in ether was evaporated to dryness in a porcelain crucible and treated with one drop of conc. H_2SO_4 containing 1% $NaNO_2$, swirled and left for a few minutes. The sample was then diluted cautiously with a drop of water. After cooling the mixture was made alkaline with 4N NaOH.

Results:

Compound	Colour formed	Colour on addn. H_2O	Colour when alkaline
Phenol	green yellow	mauve	colour disappears
C	bright yellow	brownish yellow	colour disappeared
I	bright yellow	brownish yellow	colour disappeared.
Blank	colorless	colorless	colorless.

(c) Test with 5 Nitroso 8 hydroxy Quinoline (20, 10,p.132.)

1.05 gm. of ortho hydroxy quinaldine was dissolved in 10 cc. dilute NaOH, and 0.69 gm. $NaNO_2$ added and the mixture poured into 10 cc. cold acetic acid. A deep orange solution of the free base was formed. To 5 ml. of this solution 5 ml. conc. H_2SO_4 were added.

One drop of alcoholic test solution was evaporated to dryness in a porcelain crucible and one drop of the above reagent was added to the cold residue. The crucible was then gently warmed. The test was carried out on resorcinol, C and I.

Resorcinol gave a violet red coloration, but C and I gave no coloration.

Attempted Paper Chromatogram of Compound C and 2 hydroxy 4, 6 .....(I)

On a sheet of chromatography paper 7" x 24" a line was ruled 6 cm. from one end, and this line was spotted at two points 4 inches apart with solutions of C and I.
The solution of C contained 2 mg. in 10 cc. EtOH
The solution of I contained 5 mg. in 25 cc. EtOH.

The end of the paper nearest the pencil line was inserted in a trough of solvent saturated with water, and the whole suspended in a glass box whose atmosphere was saturated with both water and solvent vapour.

The solvent used was prepared as follows:- 320 ml. n-butanol, 40 ml. glacial acetic acid and 400 ml. distilled water were mixed in a separating funnel. The upper layer of butanol rich solvent was placed in the trough of the glass box. The bottom of the box was just covered with solution from the lower layer of the separation funnel.

The chromatogram was run for 30 hours, dried in an oven and developed by spraying with a solution of 0.5 gm. diazotized sulfanilic acid in 100 cc 10% NaOH (21). This had been found to give a yellow colour with spots of both solutions in a preliminary experiment. On development the paper showed no spots, only a

SECTION V.

Installation of a high efficiency fractionating column

1. Erection of Column

A 6 ft. x 30 mm. fractional distillation column designed by the Anglo-Iranian Oil Co. Ltd., and manufactured by Griffin and Tatlock Ltd. was installed following the directions contained in the instructions accompanying the column.

The two 3'6" column sections were packed with Dixon gauze rings; the two inches at the base of each column section were packed with $\frac{1}{8}$" x $\frac{1}{8}$" rings and the remainder of both sections with $\frac{1}{16}$" x $\frac{1}{16}$" rings. Apiezon grease, No. 356 was used for all joints. The column was fitted with an automatic collection device, designed for operation under vacuum, capable of collecting 32 fractions each containing 9 ml. In order to record automatically the temperatures of the fractions withdrawn, the thermometer at the top of the column in the original design was replaced by a thermistor, the thermistor was connected to a George Kent Mk II Single point Conductivity Recorder, adapted to record the temperature. The instrument was adjusted so that the temperature range 20° - 250° was divided into the three ranges, 20-100°, 100-180°, and 180-250°; each range covered the width of the recorder, and a switch unit was installed to change from one range to another. A calibration curve was made on the recorder for the thermistor, using a calibrated thermometer and an oil bath for the lowest temperature range, and a Woods metal bath for the two higher ranges. After a preliminary calibration had been carried out, the recording equipment was adjusted to give more sensitive readings in the higher temperature regions of the first and second ranges, and the system was recalibrated. (This work was carried out jointly with Mr. J. D. Stevens.)

An apparatus was designed to maintain a constant low pressure (1-5 mm.) in the distillation system using the thermodynamic properties of n-butyl benzoate. The apparatus was based on the fact that a liquid boils at a lower temperature at a reduced pressure. The n-butyl benzoate was at the same pressure as the distillation

system, and its boiling temperature was measured by a thermister connected to an electrical relay system. As the pressure varied, the boiling temperature of the liquid varied, and a control valve was opened or closed to rectify the variation. This accessory equipment was designed and a great deal of it constructed by Dr. I. R. C. Bick.

2. Distillation of Acradenia oil on the high efficiency fractionating column.

2275 ml. of essential oil of Acradenia Franklinii were dried with anhydrous sodium sulphate and clarified by filtering through an asbestos pad. The oil was placed in a 3 l. distillation flask and distilled at ordinary pressure in the high efficiency fractionating column. It had previously been decided to cease distillation when the temperature at the top of the column reach 100°. It was found that only a small portion of the oil distilled off at ordinary pressure below 100° and also that the Audco grease, No. 356, was dissolved out by traces of moisture present in the oil, and so the distillation was discontinued after six 9 ml. fractions had been collected. It was decided not to attempt to distil the oil again until reduced pressure could be used, and the column was dismantled and cleaned as some grease had collected in the packing.

3. Preparation of n-butyl benzoate (22)

Benzoic acid (341 g.) and n-butyl alcohol (440 ml.) were placed in a 1 l. r.b. flask, and an ether soln. of boron trifluoride (215 ml.) was added. The mixture was refluxed for 50 minutes, with a drying tube filled with silica gel attached to the end of the condenser. After refluxing the flask was cooled and the unreacted acid and boron trifluoride were removed by neutralization with a 10% soln. of Na_2CO_3. The ester was extracted with ether and the solvent and excess alcohol were removed by distillation. The product plus 100 g. of commerical n-butyl benzoate were fractionally distilled on a 2 ft. column under reduced pressure P 1 mm.), with a reflux ratio of 1:6. The B.P. of the fraction collected was 88-92° according to slight variations in pressure.

DATA FOR CALIBRATION CURVE OF THERMISTER

RANGE I

Temp °C	Scale Reading	Temp °C	Scale Reading
19	3.2	66.3	65.6
21	7.6	68.5	67.1
23.5	10.5	71.1	69
27	17.8	72.4	69.7
28	19.9	73.8	70.7
29.2	22.2	75.5	71.7
30.5	24.3	76.4	72.2
32	26.7	78.6	73.6
34.5	30.4	82.5	75.5
38	35.5	85.8	77.4
41.6	40.3	88.5	78.6
44.7	43.8	91	79.7
47	46.8	92.3	80.2
48.8	48.7	94.9	81.2
50.1	50.4	96	82.2
51	51.7	100.5	83.1
52.5	53.4	102.6	83.7
54.3	55	104.9	84.3
55.8	56.6	105.2	84.6
57.5	58.3	106.5	84.9
60.2	60.5	109.6	85.6
61.7	61.8	113	86.6
63.3	63.3	117	87.5
64.8	64.2	119.5	88

RANGE 2.

Temp. °C	Scale Reading	Temp. °C	Scale Reading
99.4	2.9	150.5	72.3
103.9	11.0	152.6	74.2
106.3	15.9	155.0	76.5
108.5	20.1	158.5	79.0
111.0	24.0	162.0	81.8
112.5	26.4	163.2	82.6
113.5	28.0	165.2	83.5
116.0	31.8	166.6	84.0
119.0	36.7	168.7	85.3
122.5	41.2	170.5	86.5
124.6	44.0	172.5	87.5
126.8	47.0	175.0	89.3
128.5	48.5	180.5	91.6
128.9	49.8	183.5	94.6
130.8	52.3	184.6	94.8
133.6	55.5	186.0	95.8
136.5	58.7	189.0	96.9
140.3	62.8	191.0	98.0
144.0	66.4	192.0	98.3
146.7	69.0	192.4	98.6
149.9	71.4		

RANGE 3.

Temp°C	Scale Reading
176.1	2.1
178.7	5.0
183.9	12.7
187.9	18.7
191.6	23.6
192.8	25.9
194.1	28.1
197.4	31.5
201.4	36.3
205.0	40.8
208.0	44.4
210.0	47.4
215.2	52.2
218.7	55.6
221.0	58.0
222.2	59.5
223.1	60.2
226.0	63.7
227.8	64.4
229.6	65.7
231.3	67.4
233.0	68.7
234.1	69.7
236.0	71.6
239.8	74.2
242.2	76.1
243.9	77.3
245.0	78.5
247.1	80.1
249.7	81.7
251.3	82.8

SUMMARY

During the year, 1658 g. of essential oil of Acradenia Franklinii were collected, and the physical and chemical constants of the oil were determined.

A considerable time was spent investigating methods of extraction of Cpd. A from the oil. Extraction using Girard Reagent P is not efficient, as the yields obtained were small and not reproducible. Cpd. A apparently does not react quantitatively with the Reagent. Chromatography of the essential oil on Light's alumina gives the best yields of Cpd. A. A small quantity of Cpd. A was oxidized with $KMnO_4$ in acetone, but no identifiable products were obtained. Some additional evidence was obtained that the keto group in Cpd. A is a methyl ketone.

The structure of Cpd. C, which also occurs in the essential oil, was established as 2 hydroxy, 4,6 di-methoxy, 3 methyl acetophenone, and this compound was synthesized.

Some time was spent in assisting with the erection of a high efficiency fractionating column.

Note

All temperatures are given in ^{o}C and are uncorrected.

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